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(54) Title: METHOD AND APPARATUS FOR ISOLATING NUCLEIC ACID

(57) Abstract

     The invention features a method of isolating nucleic acid in a substantially purified form, including the steps of: a) contacting a biological sample which contains nucleic acid with a matrix comprising a solid hydrophilic organic polymer without an effective positive charge under conditions which permit the nucleic acid to bind to the matrix; and b) recovering nucleic acid from the matrix.

METHOD AND APPARATUS FOR ISOLATING NUCLEIC ACID
FIELD OF THE INVENTION

1

2   The invention relates to nucleic acid isolation, and more

3   particularly to compositions and methods useful for isolation

4   of nucleic acids.

5   BACKGROUND OF THE INVENTION

6   Nucleic acid purification from biological sources or

7   post-enzymatic reactions is frequently a primary step in

8   molecular biology studies and diagnostic tests. Many

9   techniques have been developed to isolate DNA and RNA, for

10   example, phenol extraction, alcohol precipitation, density

11   gradients, dialysis, ion exchange, electroelution, silica

12   binding, membrane filtration, and column filtration.

13   U.S. Patent No. 5,346,994 to Chomczynski discloses a

14   liquid nucleic acid isolation method employing a reagent

15   mixture of phenol, chaotropic salts and stabilizers. The

16   procedure involves cell lysis and separation of DNA, RNA and

17   proteins in different phases by centrifugation.

18   U.S. Patent Nos. 5,187,083 and 5,234,824 to Mullis

19   disclose DNA isolation methods which rely on physical trapping

20   of high molecular weight DNA on membranes, such as cellulose

21   acetate filters. These methods are designed for large DNA and

22   are not effective for generally isolating DNAs of any size or

23   molecular weight.

24   A common approach to isolating and purifying nucleic

25   acids involves binding of the negatively charged

26   phosphodiester backbone of the nucleic acid to a positively

27   charged polymer by electrostatic interactions (ion exchange).

1   U.S. Patent No. 4,935,342 to Seligson t al. discloses a

2   nucleic acid isolation method in which positively charged

3   anion exchange materials are utilized. The nucleic acids are

4   released from the matrices in solutions of high ionic strength

5   (i.e., high salt concentration). However, removal of the salt

6   is often necessary before the nucleic acids may be utilized

7   further.

8   U.S. Patent No. 5,329,000 to Woodard et al. discloses a

9   method of isolating DNA using a silicon tetrahydrazide matrix.

10   Similarly, U.S. Patent No. 5,342,931 to Woodard et al.,

11   discloses a DNA isolation method using a matrix of hydrated

12   silica. In both cases, DNA binds to the inorganic matrix and

13   is released in heated water.

14   U.S. Patent No. 4,923,978 discloses a method of isolating

15   nucleic acids using hydroxylated matrices in a column. In

16   this approach, proteins are subtracted from the biological

17   sample via adsorption onto the matrix, and nucleic acids flow

18   through the matrix. However, because this procedure is

19   subtractive, further purification and concentration of the

20   nucleic acid is often required.

21   One object of the invention is to isolate nucleic acid

22   from a biological sample in a simple, fast, and efficient

23   process that avoids heating the nucleic acid to obtain

24   elution. Another object is to provide a method which avoids

25   high speed centrifugation and phase separation to isolate a

26   nucleic acid. Another object is to provide for highly

27   quantitative recovery of nucleic acid regardless of its

28   molecular weight. Another object of the invention is to

1   isolate nucleic acid from a number of samples simultaneously,

2   thus saving time and effort and providing for subsequent

3   simultaneous processing of the samples. Yet another object of

4   the invention is to provide for simultaneous processing and

5   recovery of even small amounts of nucleic acids from multiple

6   samples. Another object of the invention is to avoid the risk

7   of loss of an isolated nucleic acid by providing a nucleic

8   acid preparation which does not require further concentration

9   from a large volume or does not require further purification.

10 Yet another object of the invention is to provide for high

11 yield recovery of nucleic acid within a broad size range.

12 Another object is to provide a method of nucleic acid

13 isolation that is environmentally friendly, i.e., that avoids

14 the required use of toxic chemicals, corrosive agents or

15 chaotropic salts.

16   SUMMARY OF THE INVENTION

17   The invention is based on a method and apparatus for

18 nucleic acid isolation. The invention utilizes the properties

19 of aggregated nucleic acids to isolate and purify nucleic

20 acids from contaminants such as other cellular components.

21 The invention is based on the discovery that aggregated

22 nucleic acid is capable of binding reversibly to a solid,

23 hydrophilic organic matrix without an effective positive

24 charge.

25   The invention therefore encompasses a method of isolating

26 nucleic acid in a substantially purified form, the method

27 comprising the steps of: a) contacting a biological sample

1   comprising aggregated nucleic acid with a matrix other than

2   cellulose or a cellulose matrix suspension under conditions

3   which permit nucleic acid in the sample to reversibly bind to

4   the matrix, the matrix comprising a solid hydrophilic organic

5   polymer without an effective positive charge; and b)

6   recovering nucleic acid from the matrix.

7   Preferably, in the recovering step, the nucleic acid is

8   eluted from the matrix in substantially purified and

9   concentrated form without heat; that is, preferably

10   substantially at room temperature or at about 80°F or less,

11   i.e., in the range of about 50-80°F or about 60-80°F. The

12   recovery step may also comprise dissociation and/or

13   solubilization of the nucleic acid aggregates in water or a

14   low salt buffer.

15   As used herein, the terms "contacting a biological sample

16   with a matrix" refers to any means known in the art of bringing

17   the nucleic acid into physical contact with a matrix as

18   described herein.

19   As used herein, "biological sample" refers to a sample of

20   biological or biochemical origin; "substantially purified"

21   refers to recovery of nucleic acid which is at least 80% and

22   preferably 90-95% purified with respect to removal of a

23   contaminant, e.g., cellular components such as protein, lipid

24   or salt; thus, the term "substantially purified" generally

25   refers to separation of a majority of cellular proteins or

26   reaction contaminants from the sample, so that compounds

27   capable of interfering with the subsequent use of the isolated

28   nucleic acid are removed; "bind to" refers to reversible

1   binding via weak forces such as Van der Waals interactions,

2   and does not refer to electrostatic interactions, affinity

3   binding or physical trapping. As used herein, "Van der Waals

4   interactions" refer to the weak forces between atoms and

5   molecules due to induced or instantaneous dipole movements,

6   which may allow packing together of organic compounds such as

7   nucleic acid and other hydrophilic organic materials (matrix).

8   "Electrostatic interactions" refer to positive (+) and

9   negative (-) charge attractions; "affinity binding" refers to

10   the sequence- or conformation-specific and directional binding

11   between a molecule and its complementary molecule; and

12   "physical trapping" refers to retaining of nucleic acid based

13   on the relatively large size of the nucleic acid and small

14   size of holes in the matrix.

15   As used herein, "without an effective positive charge"

16   refers to a matrix having a net (i.e., overall) neutral or a

17   net negative charge, or a matrix which does not possess

18   sufficient positive charge to attract, bind or retain a

19   measurable or detectable (i.e., by optical density measurement

20   at 260) amount of nucleic acid in low ionic strength buffer

21   (i.e., <10 mM of Na^+, pH 5-10). A net charge is calculated by

22   adding together the positive and negative charges in the

23   repeating units of the polymer that constitute the matrix.

24   Thus, a matrix without an effective positive charge is not

25   suitable for use as an ion exchange material for nucleic acid

26   purification by conventional methods.

27   The terms "aggregate" and "aggregation" refer to the

28   tendency of large macromolecules, such as nucleic acids, to

1 plastic material, can be obtained by modifying the backbone

2 with the addition of polar groups, including hydroxyl groups

3 (OH), carboxyl groups (COOH), amino groups (NH$_2$), groups which

4 are neutral at pH > 7, and thiol groups (SH), such that the

5 surfaces of fibrous and particulate matrices comprising this

6 polymer then possess hydrophilic properties.

7 A hydrophilic compound or a modifiable hydrophobic

8 backbone is selected from the group of polysaccharides

9 including cellulose, rayon, cellulose acetate, cellulose

10 triacetate, chitin and agarose. A hydrophilic compound also

11 may be selected from the group of protein/polypeptides

12 including leather, silk and wool. It can also be selected

13 from synthetic gels including polyacrylamide, hydrogel (i.e.,

14 copolymer of poly(vinyl alcohol) and collagen). It may also

15 be selected from the group of synthetic fibers including

16 polyamides (nylon), polyesters, polyacrylonitrile (acrylic),

17 polyurethane (spandex). It can also be selected from the

18 group of synthetic plastics including polycarbonate, phenol-

19 formaldehyde resins, polysulfide, poly(vinyl butyryl),

20 poly(vinyl chloride), poly(vinylidene chloride),

21 poly(ethylene), and polystyrene.

22 Fibrous or particulate forms of a polymer can be readily

23 prepared by mechanical means well-known in the art. "Fibrous"

24 refers to fibers of e.g., 1 micrometer - 10 micrometers and

25 as long as 100, 1,000 or 10,000 micrometers; and "particulate"

26 refers to particles of e.g., about 1 micron to 5 microns, or

27 even as large as 10, 50, or 100 microns in diameter. Some

28 examples of fibrous cellulose currently on the market include

1   Sigma CF11 cellulose catalog # C6288. An example of

2   particulate cellulose currently on the market is microgranular

3   cellulose Sigma C-6413 and product number CM1000, Megacell,

4   sold by Cortex Biochemicals, San Leandro CA. Particulate

5   cellulose includes any microgranular substance coated with

6   cellulose, e.g., cellulose-coated magnetic beads.

7   Preferably, the aggregated nucleic acid binds to a matrix

8   suspension.

9   As used herein, "matrix suspension" refers to an insoluble

10   matrix immersed in a liquid such that free-floating pieces of

11   the matrix can move freely relative to the container and

12   relative to other free-floating pieces of matrix when the

13   container is shaken or when the liquid is stirred. One

14   example of a matrix suspension is cellulose fibers immersed in

15   liquid at a density such that the liquid appears visually

16   turbid when the container is shaken, and appears to clear when

17   the cellulose fibers settle to the bottom of a stationary

18   container. That is, when the container is shaken, the

19   cellulose fibers move freely relative to the container and to

20   each other. A matrix suspension does not refer to a

21   suspension of a support material having an immobilized matrix

22   attached thereto. However, the invention may encompass a

23   suspension of sets of immobilized cellulose fibers, each set

24   being cellulose fibers attached to each other indirectly via a

25   support, and each set moving freely relative to other sets

26   upon movement of the immersing liquid. That is, when shaken,

27   all sets move freely relative to the container and each other,

28   and cellulose fibers within a set have a fixed range of

1   movement r lativ to each other.

2   In contrast, a matrix which is not in suspension is a

3   solid material packed into a container, for example, into a

4   cylinder or conical-shaped container that may remain at both

5   ends such that only the liquid, and not the solid materials,

6   moves freely through the container. A typical non-suspension

7   cellulose matrix is known in the art as a "column" containing a

8   packed cellulose matrix. This type of column can retain

9   nucleic acid by physical entrapment within the cellulose

10   matrix. A column containing a packed matrix is not to be

11   confused with a column containing a suspension matrix. The

12   key to distinguishing a matrix suspension from a non-matrix

13   suspension is the freedom of movement of the matrix relative

14   to other particles of matrix or relative to the container.

15   A "matrix-collection" device also is useful according to

16   the invention and refers to a cylinder or conical-shaped

17   container that is open at both ends to liquid movement and fit

18   with a barrier at one end to prevent solid materials from

19   passing through.

20   In preferred embodiments, the method further includes the

21   initial step of contacting the biological sample with a buffer

22   under conditions to solubilize the nucleic acid, i.e., to

23   dissolve the nucleic acid. These conditions include

24   resuspension of the nucleic acid in an aqueous (e.g. Tris-

25   EDTA) buffer, contacting the biological sample in a detergent

26   buffer with a proteolytic enzyme under conditions sufficient

27   to subject the sample to proteolysis and release of nucleic

28   acid, contacting the biological sample with chaotropic agents,

1   or other m thods known in the art to release nucleic acids

2   from cellular components into solution.

3   The method further includes the step of aggregating the

4   nucleic acid, wherein the nucleic acid is aggregated by

5   contacting the biological sample with a precipitant selected

6   from the group consisting of organic solvents, soluble organic

7   polymers and salts, (and combinations thereof) wherein the

8   organic solvent may be any one of isopropanol, ethanol,

9   acetone, and organic polymers including but not limited to

10   polyethylene glycol (PEG), and wherein the salt may include

11   but is not limited to NaCl and LiCl. Co-precipitants, such as

12   glycogen, also may be used to facilitate the precipitation of

13   nucleic acid present in only small quantities; for example, in

14   the form of heteroaggregates. The presence of a co-

15   precipitant is not required according to the invention, but

16   serves to increase the efficiency of aggregate formation.

17   Small molecules and digested proteins do not bind to the

18   matrices and thus may be separated from the nucleic acid by

19   washing the adsorbed, aggregated nucleic acid. Therefore, the

20   method may also include a washing step using solutions that

21   contain a precipitant at a concentration sufficient to

22   maintain a nucleic acid in aggregated form.

23   The invention also includes an apparatus for isolating

24   nucleic acid in a substantially purified form from multiple

25   biological samples simultaneously, the apparatus comprising:

26   plural housings for isolation of nucleic acid from plural

27   biological samples, wherein each housing comprises an inlet

28   and an outlet and defines a flowpath for flow of a biological

1 sample therethrough, the flowpath comprising a matrix

2 comprising a solid hydrophilic organic polymer without a net

3 positive charge, and support means for holding plural housings

4 in place such that nucleic acid in plural biological samples

5 may be handled and isolated simultaneously.

6   Preferably, each housing of the apparatus further

7 includes a barrier means to allow flow of liquid along the

8 flowpath and through the housing outlet, but to prevent the

9 matrix from exiting the housing via the outlet. Most

10 preferably, the housing comprises a lower portion leading to

11 the outlet and the barrier is positioned within the lower

12 portion of the housing. The apparatus may also include means

13 for connecting the plural housings to a vacuum, magnetic or

14 pressure source. The barrier may be any material which

15 prevents the matrix from exiting the housing but which allows

16 liquid comprising nucleic acid to flow through and exit the

17 housing, for example, a mesh screen, cotton fibers, synthetic

18 fibers, tissue paper, or siliconized glass fibers. The

19 apparatus may also include a collection tray for

20 simultaneously collecting waste or plural nucleic acid

21 samples. In a preferred embodiment, the apparatus may be

22 subjected to vacuum force, pressure force or low

23 centrifugation force. In another embodiment, the apparatus is

24 constructed such that it does not allow for cross-

25 contamination of the samples.

26   The invention also encompasses an apparatus comprising a

27 matrix-coated surface, for example, a flat surface onto which

28 a sufficient amount of cellulose is coated so as to permit

1 binding of aggregated nucleic acid to the surface, e.g., a

2 cellulose-coated plastic or glass column or microtiter dish.

3   The method and apparatus of the present invention permits

4 the isolation of nucleic acid having essentially any molecular

5 weight or form (i.e., circular, linear, etc.) in a rapid and

6 high-yield manner. The apparatus allows for such isolation

7 from plural samples simultaneously.

8   The nucleic acid isolated as described herein may be of

9 any molecular weight and in single-stranded or double-stranded

10 form; i.e., small oligonucleotides such as 10 - 50 bases in

11 length, small nucleic acid fragments of, for example, 100

12 bases - 500 bases in length, or relatively longer fragments of

13 1000 bases - 10,000 bases in length. Alternatively, high

14 molecular weight nucleic acid, e.g., 50 kb-500 kb may be

15 isolated as described herein. Preferably, a nucleic acid

16 isolated according to the invention will be in the range of 50

17 bases to 500 kilobases.

18   The nucleic acid sample applied to the matrix according

19 to the methods described above may be in any convenient

20 volume. Where large-scale isolation is contemplated, the

21 applied volume may be correspondingly large, e.g., 1 liter,

22 500 ml, 100 ml, 50 ml, etc. Alternatively, where other than

23 large-scale isolation is contemplated, a correspondingly

24 smaller volume may be applied to the matrix, e.g., less than

25 50 ml, more preferably, less than 5 ml, less than 500 µl;

26 e.g., 1-100 µl.

27   The volume of nucleic acid isolated according to the

28 invention may be in any selected volume which is sufficient to

1   saturate the matrix. For example, for nucleic acid isolation

2   on a large-scale, the volume of isolated nucleic acid may be

3   correspondingly large, e.g., 1 - 100 ml, as described above

4   for the applied volume. Alternatively, the isolated nucleic

5   acid may be recovered in a smaller volume, e.g., less than 500

6   µl, 250 µl, 100 µl; e.g., 1-50 µl.

7   The nucleic acid applied to the matrix, as described

8   herein, may be any amount, that amount being determined by the

9   amount of matrix. Preferably, the amount of nucleic acid (and

10   plus co-precipitant, if desired) applied to the matrix is less

11   than the dried weight of the matrix, typically in the range of

12   1/10,000 to 1/10 (weight nucleic acid/matrix). The amount of

13   nucleic acid applied to the matrix may be as much as 100 gm or

14   as little as a few molecules. Preferably, the amount of

15   nucleic acid applied to the matrix is less than a total of 100

16   mg, more preferably in the range of 10 mg-0.1 ng, and most

17   preferably, in the range of 500 µg-1 ng.   The nucleic acid

18   isolated from the matrix will generally be in an amount which

19   is about 90% or more than the amount of nucleic acid applied

20   to the matrix.

21   The invention is particularly useful in procedures

22   wherein large numbers of samples are handled simultaneously,

23   for example, in newborn screening, where as many as 4-5

24   million samples of newborn blood nationwide are analyzed

25   annually. Molecular screening of newborns is still in its

26   infancy and large scale screening is still difficult, mainly

27   due to lack of a suitable nucleic acid purification method.

28   The invention provides for easy simultaneous recovery of

1   plural nucleic acid samples. In addition, because the

2   inventive methods and devices do not allow for leaking between

3   sample chambers, the danger of cross-contamination of samples

4   is significantly reduced throughout the isolation procedure,

5   i.e., before, during and after isolation of the sample from

6   the sample matrix. These advantages are especially important

7   for uses contemplated in the invention, i.e., where hundreds

8   of individual samples, e.g., 100, 200-500, or thousands, e.g.,

9   1000, 2000, 3000, 4000-6000, or even up to ten thousand or

10   one-hundred thousand individual samples are analyzed

11   simultaneously.

12   The invention thus provides for high yield recovery of

13   relatively pure nucleic acid molecules from a biological

14   sample. The nucleic acid may be recovered efficiently from a

15   number of samples simultaneously, thus saving time and effort

16   and providing for subsequent simultaneous processing or

17   analysis of numerous nucleic acid samples, if desired. Any

18   number of samples may be subjected to isolation simultaneously

19   according to the invention, e.g., a single sample, two

20   samples, tens of samples, 100's and even thousands of samples

21   are conveniently isolated using the devices and methods

22   disclosed herein. The invention thus provides for isolation

23   of nucleic acids from hundreds or thousands of samples

24   routinely in an efficient and safe manner. The number of

25   simultaneously isolated samples is only limited by the number

26   of sample chambers which are present in a single format. A

27   format may include a single planar surface containing numerous

28   individual sample chambers or it may include multiple

1   surfaces; the latter format would include multiple stacked

2   surfaces or multiple side-by-side surfaces.

3   Nucleic acids may be selectively recovered from impure

4   samples such as body fluids, cells, tissues or other types of

5   biological samples according to the invention. Exceedingly

6   small amounts of nucleic acid molecules may be simultaneously

7   and quantitatively recovered according to the invention. For

8   example, 80-90% of the small amount of DNA or RNA present in

9   the dried blood spot samples that are routinely assayed in

10   newborn screening (e.g., equivalent to 15 µl newborn blood or

11   about 0.4 µg DNA) can be recovered. The yield of recovery is

12   mainly dependent on the quality of the dried blood sample

13   rather than the procedure itself. Because the invention

14   provides a nucleic acid preparation that does not require

15   further concentration from a large volume, the invention

16   avoids risk of loss of the isolated nucleic acid.

17   Nucleic acids isolated according to the invention, will

18   be useful, for example, in assays for detection of the

19   presence of a particular nucleic acid sequence in a sample.

20   Such assays are important in the prediction and diagnosis of

21   disease, forensic medicine, epidemiology and public health.

22   For example, isolated DNA may be subjected to hybridization

23   and/or amplification to detect the presence of an infectious

24   virus or a mutant gene in an individual, allowing

25   determination of the probability that the individual will

26   suffer from a disease of infectious or genetic origin. The

27   ability to detect an infectious virus or a mutation in one

28   sample among the hundreds or thousands of samples being

1   screened takes on substantial importance in the early

2   diagnosis or epidemiology of an at-risk population for

3   disease, e.g., the early detection of HIV infection, cancer or

4   susceptibility to cancer, or in the screening of newborns for

5   diseases, where early detection may be instrumental in

6   diagnosis and treatment.

7   In addition, the method can also be used in basic

8   research labs to isolate nucleic acid from cultured cells or

9   biochemical reactions. The purified nucleic acid can be used

10   for enzymatic modification such as restriction enzyme

11   digestion, sequencing and amplification.

12   Further objects and advantages of the invention will be

13   apparent in light of the following description and the claims.

14   BRIEF DESCRIPTION OF THE FIGURES

15   Before describing the invention in detail, the drawings

16   will be briefly described.

17   FIG. 1 is a diagram of an embodiment of the invention

18   employing a sample collection tray; and

19   FIG. 2 is a diagram of another embodiment of the

20   invention adapted for connection to a vacuum source.

21   DETAILED DESCRIPTION OF THE INVENTION

22   The invention encompasses a method and apparatus for

23   nucleic acid isolation and concentration, and takes advantage

24   of the discovery that aggregated nucleic acid binds reversibly

25   to a solid, hydrophilic organic matrix without an effective

26   positive charge.

1   The invention utiliz s the properties of aggregated

2   nucleic acid to isolate and separate nucleic acid from other

3   biochemical or cellular components such as heparin, which

4   tends to inhibit sensitive enzymatic or chemical reactions

5   such as PCR. Nucleic acid can be released in aqueous buffer

6   from cells or tissues by essentially any known method, such as

7   mechanical disruption, sonication, detergent solubilization,

8   treatment with chaotropic agents, and the like. Once released

9   from cells or tissues and separated from insoluble materials,

10 nucleic acid in solution is allowed to form aggregates in the

11 presence of precipitants.

12   According to the invention, a nucleic acid aggregate is

13 contacted with a solid, hydrophilic organic matrix without an

14 effective positive charge under conditions and for a time

15 sufficient to allow it to bind reversibly to the matrix. If

16 desired, the matrix-nucleic acid complex may be washed to

17 remove contaminants, then dissociated and/or solubilized, and

18 nucleic acid recovered in water or low salt buffer without

19 heat. The method of the present invention permits the

20 investigator or technician to isolate nucleic acid of

21 essentially any molecular weight in a rapid, high-yield

22 manner.

23   The nucleic acid aggregate binds to the matrix suspension

24 as a result of contacting the sample containing the aggregated

25 nucleic acid with the matrix suspension. The matrix

26 suspension may be contained in a column, and thus, as the

27 sample is passed throughout the matrix suspension, the nucleic

28 acid becomes bound to the hydrophilic non-net-positively

1   charged surface of the matrix. The matrix suspension also may

2   be contained in a conventional tube, dish or well, such that

3   the sample is mixed with the matrix, rather than passed

4   through it. Alternatively, the sample may be applied to a

5   matrix which is attached to a surface, e.g., mesh, beads, a

6   plate, a column, or the like. In this embodiment, the sample

7   is passed over the matrix for the nucleic acid to bind.

8   Some examples of matrix formats useful according to the

9   invention include passing the aggregated nucleic acid through

10   a column containing a matrix suspension, contacting the

11   aggregated nucleic acid with free fibers in suspension,

12   contacting the nucleic acid with fibers that are attached to a

13   support, whether the support be a mesh, a bead, a plate well,

14   or the like.

15   Where the matrix is cellulose, the invention contemplates

16   a matrix suspension of cellulose rather than a packed

17   cellulose column, as it has been discovered that a cellulose

18   matrix suspension is superior to a packed cellulose column in

19   binding nucleic acid.

20   However, non-cellulose matrices according to the

21   invention may be utilized in a packed matrix column if the

22   efficiency of binding is not at least two-fold better in

23   suspension than in a packed column format.

24   Once the nucleic acid binds to the matrix, digested or

25   solubilized proteins and salts do not bind and thus are

26   separated from nucleic acid in that they flow through the

27   matrix. The bound nucleic acid is eluted from the matrices

28   and recovered in a substantially pure and concentrated state,

1   suitable for direct use.

2   Solid hydrophilic organic polymers that constitute a

3   matrix useful according to the invention fall within the

4   definition provided hereinabove. A matrix according to the

5   invention will include any solid, hydrophilic organic matrix

6   without an effective positive charge that reversibly binds

7   nucleic acid substantially by weak forces such as Van der

8   Waals interactions and not by electrostatic interactions,

9   affinity binding, or physical trapping. Preferably, the

10   matrix is essentially neutral, i.e., without any positive or

11   negative charge.

12   The term "solid matrix", as used herein, encompasses a

13   polymer that is substantially insoluble in water and alcohol

14   at less than about 50 degrees centigrade. Preferably, a solid

15   matrix is in particulate form, with the particles being in the

16   micro-meter range (preferably, 5-500 μmeters) or the milli-

17   meter range (preferably, 0.1-10 millimeters); or is in fibrous

18   form with the fibers being micro-meter in diameter and of any

19   desired length.

20   The term "polymer" includes matrices made from repeating

21   units of two or more monomer repeats. As used herein,

22   "polymer" also includes homopolymers and heteropolymers, a

23   "homopolymer" being defined as a polymer consisting

24   essentially of repeating units of identical monomers, and a

25   "heteropolymer" being defined as a polymer consisting

26   essentially of two or more monomers which are not identical,

27   the monomers being repeated in a given order or randomly. A

28   "mixed polymer" is defined herein as including two or more

1   homopolym rs or heteropolymers, or a combination of a

2   homopolymer and a heteropolymer.

3   Exemplary monomer materials include acrylonitrile,

4   acrylene, caprolactam, chloroprene, dichloroethene, ethylene,

5   isoprene, propylene, tetrafluoroethene, vinyl chloride,

6   vinylidene fluoride, acrylamide, amino acids, diisocyanate,

7   divinylbenzene, ethylene glycol, formaldehyde, glycol, methyl

8   methacrylate, styrene, sugars, terephtalic acid. Additional

9   exemplary polymer materials include but are not limited to

10 polysaccharides including cellulose, rayon, cellulose acetate,

11 cellulose triacetate, chitin and agarose; protein/polypeptides

12 including leather, silk and wool; synthetic gels including

13 polyacrylamide, hydrogel (i.e., copolymer of poly(vinyl

14 alcohol) and collagen); synthetic fibers including polyamides

15 (nylon), polyesters, polyacrylonitrile (acrylic), polyurethane

16 (spandex); and synthetic plastics including polycarbonate,

17 phenol-formaldehyde resins, polysulfide, poly(vinyl butyryl),

18 poly(vinyl chloride), poly(vinylidene chloride),

19 poly(ethylene), and polystyrene.

20   The present invention does not utilize ion-exchange

21 procedures, affinity binding, density gradients,

22 aqueous/organic phase separation, or physical trapping to

23 separate the nucleic acids from other cellular or tissue

24 components. Thus, subsequent removal of large amounts of

25 salt, as is present in samples having been prepared by such

26 methods, or further purification of nucleic acids, as is

27 necessary for samples having been prepared using gradients, is

28 not necessary. Moreover, the method of the invention is fast

1   and the yield of recovery for large siz s of nucleic acids is

2   superior to other methods of the prior art.

3   The invention features methods and devices for the

4   efficient and quantitative recovery of relatively small

5   amounts of nucleic acid from single or plural samples by

6   binding to a matrix having the characteristics described

7   herein.

8   The nucleic acid to be isolated can be present in any

9   type of biological sample, and will generally be a sample of

10   medical, veterinary, forensic, environmental, nutritional,

11   scientific or industrial significance. Human and animal

12   specimens and body fluids particularly can be assayed by the

13   present method, providing that they contain cells, or

14   particles, e.g., virions, from which nucleic acid can be

15   prepared. Preferred sources include blood, sperm, any

16   mammalian tissue, milk, urine, cerebrospinal fluid, sputum,

17   fecal matter, and lung aspirates, all of which may have been

18   collected as is or previously adsorbed onto a fluid collection

19   device such as a swab; in addition, buccal cells, throat

20   swabs, genital swabs and exudates, rectal swabs, and

21   nasopharyngeal aspirates.

22   The invention allows for simultaneous recovery of

23   exceedingly small amounts of nucleic acid from, e.g., hundreds

24   of a type of sample in a quantitative manner. Typically 70-

25   100%, and more likely at least 80%, 90% or most likely at

26   least 95%, of the nucleic acid that is present in a biological

27   sample may be recovered according to the invention, even when

28   the sample contains such small amounts as less than 1 ng of

1   nucleic acid. For example, as much as 0.3-0.4 μg of genomic

2   DNA may be recovered from a 15 μl dried blood spot according

3   to the invention. Because the invention allows for recovery

4   of the isolated nucleic acid into a relatively small volume of

5   liquid, e.g., smaller than 500 μl, 250 μl, 100 μl, and even as

6   small as 5-10 μl, the invention avoids the risk of loss of the

7   recovered nucleic acid encountered in concentrating a sample

8   from a relatively large volume.

9   The nucleic acid may be isolated or concentrated

10   according to the invention from an impure, partially pure, or

11   a pure sample. The purity of the original sample is not

12   critical to the inventive methods, as nucleic acid may be

13   isolated from even grossly impure samples according to the

14   invention. For example, nucleic acid may be removed from an

15   impure sample of a biological fluid such as blood, saliva, or

16   tissue. If a sample of higher purity is desired, the sample

17   may be treated according to any conventional means known to

18   those of skill in the art prior to undergoing isolation

19   according to the invention, e.g., the sample may be processed

20   so as to remove certain impurities such as insoluble materials

21   from an impure sample prior to nucleic acid isolation.

22   Methods of the invention may be performed on a biological

23   sample which has been deposited on any type of material,

24   provided the material itself does not form cross-linkages to

25   retain the nucleic acid irreversibly. Thus, the sample may be

26   contained within a material such as paper, textile, e.g., a

27   fragment of an article of clothing, agarose, or

28   polyacrylamide. One example of a sample and sample matrix

1   according to the invention is a drop of a body fluid, e.g.,

2   blood, which has been stored dry on a piece of Schleicher and

3   Schuell #903 paper, the paper routinely used nationwide for

4   the purpose of newborn screening.

5   For purposes of the following description of the

6   invention, recovery of nucleic acid in the form of genomic

7   DNA, plasmid DNA, or single-stranded DNA or RNA is described

8   in detail herein. However, it is to be understood that the

9   invention encompasses recovery of any form, whether

10   fragmented, circular, single stranded (RNA and some

11   bacteriophage and virus DNAs and RNAs) or chromosomal DNA.

12   The method of the invention is applied to isolation of

13   nucleic acid from a biological sample, as follows.

14   Tissues or cells that contain nucleic acids are suspended

15   in an extraction solution that contains a buffer system, a

16   detergent, and a chelating agent. The buffer system can be

17   any buffer, e.g., TrisHCl, sufficient to maintain pH values

18   from approximately 5.0 to approximately 10. The detergent can

19   be ionic or nonionic detergent, such as sodium dodecyl sulfate

20   (SDS) or octylglucoside, at a concentration sufficient to lyse

21   cells and denature proteins. A chelating agent, such as EDTA,

22   captures free divalent ions (Mg^{2+}) so that nucleic acids are

23   more soluble and protected from degradation by DNA-degrading

24   enzymes that require Mg^{2+}.

25   A protease may also be added to the extraction mixture to

26   digest proteins so that the nucleic acids can be easily

27   released from the cells and so that the proteins are degraded

28   to small peptides and become more soluble in solution. Any

1   non-specific or specific prot ase may be us d, for example,

2   proteinase K, trypsin, chymotrypsin, or V8 protease.

3   Nucleic acid aggregates are next formed by precipitating

4   the nucleic acid. Structurally, nucleic acid possesses a

5   phosphodiester backbone that is negatively charged around

6   neutral pH. Nucleic acid becomes insoluble (i.e.,

7   precipitated) in the presence of salts and agents that can

8   reorganize its aqueous environment. Acetone, alcohols such as

9   ethanol and isopropanol, and soluble organic polymers, such as

10   polyethylene glycol (PEG) are examples of useful precipitants.

11   Co-precipitants, such as glycogen, also may be used to

12   facilitate the precipitation of nucleic acid present in only

13   small quantities; for example, in the form of

14   heteroaggregates. The presence of a co-precipitant is not

15   required according to the invention, but serves to increase

16   the efficiency of aggregate formation. In the presence of

17   monovalent salt, the charges in nucleic acids are neutralized

18   so that nucleic acid aggregates are formed and stabilized by

19   weak forces such as Van der Waals forces. Divalent salts such

20   as magnesium chloride or calcium chloride can also be used to

21   precipitate nucleic acids.

22   As indicated above, the matrix may be any matrix that

23   reversibly binds nucleic acid. In preferred embodiments

24   described in detail herein, cellulose, agarose powder, and

25   polyacrylamide are used as matrices. Where the matrix is

26   fibrous, the fibers may be mechanically broken to 1 to 2 mm

27   fibers. The matrix is washed to remove salts and other

28   contaminants. The matrix can either be stored dry or in an

1   aqu ous solution at room temperature in a concentration

2   convenient for use. The matrix is in suspension and may be

3   used in a column format, for example, in a pipet tip, syringe,

4   or larger column containing a matrix suspension.

5   Alternatively, the matrix may be coated onto a well or an

6   inorganic microparticle 3-4 microns, such as a magnetic or

7   silicon bead, such that the nucleic acid binds to the matrix

8   coating on the particle. This embodiment is advantageous over

9   the isolation of nucleic acid via physical entrapment by

10   uncoated microparticles, as the purity and yield of the

11   nucleic acid is better.

12   Once the matrix is prepared, the matrix is added to the

13   suspension containing the nucleic acid before or after

14   aggregation, or the nucleic acid aggregate suspension is

15   allowed to bind to the matrix in the column. Unbound

16   materials, such as digested proteins, lipids, and other

17   unwanted cellular components are then separated from the bound

18   nucleic acid aggregates by retaining the nucleic acid/matrix

19   complexes in a column, filter, tube, or plate well. In one

20   embodiment, when the matrix is in a column format, the bound,

21   aggregated nucleic acids may be purified by washing the column

22   with a buffer to wash away the undesirable materials. Once

23   these materials are removed, the aggregated nucleic acid may

24   be recovered by eluting them from the matrix material after

25   dissolving the aggregates in water or low salt buffer. The

26   recovered nucleic acid is substantially pure, concentrated,

27   and suitable for immediate use in subsequent experiments.

28   The invention is better illustrated with respect to the

1   following examples. Th se examples are meant to be

2   illustrative of carrying out the invention, and not to be

3   limiting with respect to the spirit and scope of the invention

4   and the claims.

5   EXAMPLE 1

6   Preparation of Cellulose Matrix and Experimental Conditions.

7   Materials and reagents used in this Example and in

8   general (unless otherwise specified) are as follows:

9   1. TE buffer (10 mM TrisHCl, 1 mM EDTA, pH 8).

10   2. Matrix solution: 50 mM TrisHCl, pH 8.0, 10 mM EDTA.

11   3. Extraction buffer: 1% SDS, 50 mM TrisHCl pH 8, 10 mM

12   EDTA, 150 mM NaCl, 20 ug glycogen/ml, 50 ug/ml proteinase k.

13   4. Washing solution; 60% ethanol in buffer which

14   contains 200 mM NaCl, 50 mM TrisHCl, 5 mM EDTA.

15   5. Elution buffer: 5 mM TrisHCl pH 9, 0.5 mM EDTA.

16   6. 40% PEG in water.

17   7. 20% PEG in 2.5 M NaCl.

18   8. 5 M NaCL.

19   9. GEDTA: 120 g of GuSCN in 100 ml of 0.2 M EDTA at pH

20   8.

21   10. Isopropanol (precipitant).

22   11. Co-precipitant: glycogen (5 µg/µl).

23   12. TBE buffer: 45 mM Tris-Boric acid and 1 mM EDTA.

24   13. Whatman cellulose: 3MM paper is cut and dissociated

25   into fibers with an average length of 1-2 mm (length is not

26   critical). This is the matrix used in the following

27   experiments unless otherwise specified.

1   14. Sigma cellulos : Fibrous medium (C6288=CF-11), washed

2   to remove small particles and resuspended in TE buffer (10 mM

3   TrisHCl, 1 mM EDTA, pH 8) at 10 mg/ml.

4   15. Control DNA: Sigma, Human placenta DNA (D7011),

5   dissolved in TE buffer at 0.26 ug/ul.

6   16. Whole blood: anticoagulated in EDTA. Aliquotted for

7   use as 250-ul-samples and stored at -70°C.

8   17. Matrix collection device: AP-200 (P-1000) tip with a

9   barrier made up of a folded Kimwipe paper (1/4" disk for P-

10 200, 10x10 mm for P-1000 tip). Such a device is not able to

11 retain nucleic acid under conditions to be described.

12 18. All experiments are carried out at room temperature

13 unless otherwise specified.

14 19. Centrifugation is performed in a standard Ependorf-

15 type microcentrifuge.

16 Matrix is prepared by the following method unless

17 otherwise specified. Filter paper consisting essentially of

18 cellulose (0.25 g of Whatman 3 MM paper) is cut into < 4 mm^2

19 pieces and suspended in about 10 ml of matrix solution,

20 followed by vigorous vortexing until the paper becomes

21 fibrous. The suspension is filtered through a membrane with

22 0.45 micron or larger pores to remove liquid and the fibers

23 are recovered from the filter. The wet fibers are then

24 subjected to the procedure of cutting-vortexing-filtration

25 twice so that all cellulose fibers are dissociated. The

26 fibrous matrix thus formed is stored in either 50 ml matrix

27 solution or 50 ml washing solution at room temperature (5

28 mg/ml). When stored in this manner, the fiber suspension is

1   free to pass through a pipet with 2 mm opening. The volume

2   that the 0.25g matrix occupies after settling is equivalent to

3   10 ml-12.5 ml.

4   Matrix-collection device: A cylinder or conical-shaped

5   container that is open at both ends to liquid movement and fit

6   with a barrier on one end to prevent solid materials from

7   passing through.

8

9   EXAMPLE 2

10   Isolating DNA from Dried Blood Spots on Filter Paper

11   Using Cellulose Matrix.

12   Solutions and reagents are the same as those used in

13   Example 1 unless otherwise specified. In addition, 1 M $MgCl_2$

14   and chelating resin are used. Matrix suspensions are prepared

15   as in Example 1 unless otherwise specified.

16   4 full circles (15 mm diameter) of dried blood spots are

17   removed from S&S 903 filter papers. Two of them are immersed

18   in 5 ml of extraction buffer (Sample 1) and the other two are

19   placed in 5 ml of the same buffer plus 0.1 g chelating resin

20   (Sample 2). The samples are incubated at 56°C for about 2

21   hours to digest proteins by proteinase K.

22   a.   Phenol-extraction and ethanol precipitation (control
23   method):

24   1.   500 µl of Sample 1 (designated 1-0) and 500 µl of

25   Sample 2 (designated 2-0) are extracted with 500 µl of

26   phenol:chloroform twice.

27   2.   Glycogen is added to 20 µg/ml and NaCl is added to

1   0.1 M.

2   3.   1 ml ethanol is mixed with the extracted samples to

3   precipitate nucleic acids at -20°C for 2 hours.

4   4.   Precipitated nucleic acids are collected by

5   centrifugation for 15 min at 12 k rpm and finally dissolved in

6   50 µl of water.

7   B.   Matrix method (method of the invention):

8   1.   4 aliquots of 500 µl (1-1 to 1-4) are retrieved from

9   Sample 1, and the same for Sample 2 (2-1 to 2-4). NaCl was

10   added to 0.1 M in each.

11   2.   The samples are mixed with co-precipitants: glycogen

12   (10 µg in 1-1, 1-2, 2-1, 2-2) and Mg^{2+} (final concentration 20

13   mM in 1-1, 1-3, 2-1 and 2-3).

14   3.   Isopropanol (650 µl) is mixed with each sample and

15   the mixtures are incubated at room temperature for 20 min to

16   precipitate nucleic acids.

17   4.   Precipitated nucleic acids are loaded onto pre-

18   equilibrated matrix (5 mg matrix each) connected to a vacuum

19   manifold unit.

20   5.   The matrix are washed twice with 1 ml washing

21   solution under vacuum and dried by centrifugation at 5 k rpm

22   for 1 min.

23   6.   50 µl of elution buffer is added to each dried

24   matrix to dissolve nucleic acids at room temperature for 5

25   min. The nucleic acids in a sample are recovered into a 1.5

26   ml tube by centrifuging the tubes at 7 k rpm for 2 min.

27   5 µl of each control sample (1-0 and 2-0) and the samples

28   purified by columns are separated in 1% agarose gel containing

1   0.5 μg/ml of ethidium bromid by a standard method.

2   The same amount of nucleic acid (mainly DNA) is recovered

3   by the matrix method as by the control method, based on gel

4   electrophoresis analysis. Samples treated with chelating

5   resins contained DNAs of relatively large molecular weights.

6   DNA isolated from filter paper by the nucleic acid

7   isolation method described herein is consistently found to be

8   compatible with DNA modifying enzymes. The DNA thus-isolated

9   is also digestible by restriction enzymes such as ScrF1, and

10   can be used for polymerase chain reaction (PCR).

11   Thus, high yield isolation of nucleic acid from small

12   quantities of clinical samples may easily be achieved using

13   the method of the invention. Because high speed

14   centrifugation (>10,000 rpm) is not required, the method can

15   be easily automated. The total isolation time for solubilized

16   nucleic acids may be shorter than 30 min.

17   EXAMPLE 3

18   Isolation of Plasmid DNA Using Cellulose Matrix.

19   Reagents, matrix and columns are the same as in Example 1

20   unless otherwise specified. In addition, a plasmid isolation

21   kit (Wizard Kit) from Promega was used for comparison. LB

22   media used in this example contained 1% tryptone, 0.5% yeast

23   extract and 1% NaCl in water.

24   Source of double-stranded plasmid DNA: E. coli cells

25   harboring pBluescript plasmid (Stratagene) are grown overnight

26   in LB media containing ampicillin (100 μg/ml).

27   Source of single-stranded plasmid DNA: E. coli cells

28   harboring pBluescript plasmid (Stratagene) are infected by

1   M13K07 helper phages (NEB) to generate single-stranded plasmid

2   DNA. The cells are grown overnight at 37°C in LB media

3   containing ampicillin (100 µg/ml) and kanamycin (70 µg/ml).

4   To purify double-stranded plasmid DNA, the following

5   steps are performed:

6   1.   An overnight culture is split into 6 aliquots of 1-

7   ml samples (a-1 to a-6).

8   2.   Crude plasmid DNA is obtained by alkaline lysis

9   method with reagents in the Wizard plasmid isolation kit,

10   following instruction from the manufacturer.

11   3.   DNAs in samples a-1 to a-3 are further purified with

12   silica powder provided in the same Wizard kit. Each DNA

13   sample is finally recovered in 50 µl of water.

14   4.   Other crude DNA samples (a-4 to a-6) are mixed with

15   750 µl of isopropanol and incubated for 20 min at room

16   temperature.

17   5.   Precipitated DNAs are mixed with 5 mg matrix and

18   prepared as described in Example 1.

19   6.   Washing and DNA recovering are the same as described

20   in Example 1 and 2. DNA in each column is eluted in 50 µl of

21   elution buffer.

22   To purify single stranded plasmid DNA, the following

23   steps are performed:

24   1.   8 clear supernatants of 1 ml (B-1 to B-8) are

25   recovered from a helper phage-infected culture after removal

26   of cells by centrifugation.

27   2.   250 µl of PEG solution (20% polyethylene glycol-8000

28   plus 2.5 M NaCl) is mixed with each of the 8 samples. The

1   solutions ar k pt at room temperature for 15 min.

2   3.   Precipitated phage particles are harvested by

3   centrifugation at 10k rpm for 5 min at room temperature.

4   4.   After complete removal of the liquid, 500 µl of

5   extraction buffer is added to each pellet and incubated at

6   56°C for 40 min to release DNA.   NaCl is then added to 0.2 M.

7   5.   4 samples (B-1 to B-4) are subjected to

8   phenol:chloroform extraction twice followed by ethanol

9   precipitation as described in Example 2.   The DNA pellets are

10 dissolved in 50 µl of TE buffer.

11   6.   The other 4 samples (B-5 to B-8) are mixed with 10

12 µg glycogen and 625 µl of isopropanol, followed by

13 purification with matrix as described in Example 1.   Each DNA

14 sample for recovery is dissolved in 50 µl of elution buffer.

15   5 µl of each purified DNA is separated in a 1% agarose

16 gel containing 0.5 µg/ml ethidium bromide for analysis.

17   The results demonstrate that similar amounts of double-

18 stranded plasmid DNAs are isolated with cellulose matrix as

19 with a silica matrix.   Single-stranded plasmid DNAs are also

20 isolated, although the amount of DNA isolated by the cellulose

21 matrix method is slightly less than that isolated after phenol

22 extraction and ethanol precipitation.   DNAs isolated by the

23 cellulose matrix method are sequenced as efficiently as DNAs

24 isolated by phenol extraction and ethanol precipitation

25 method.   Plasmid isolation by this method of the invention

26 eliminates the requirement for chaotropic agents and minimizes

27 the use of high speed centrifugation.

1   EXAMPLE 4

2   RNA Isolation Using Cellulose Matrix.

3   Solutions and reagents are the same as in Example 1

4   unless otherwise specified.

5   RNA may be isolated by the following steps:

6   1.   1 g of fresh and soft plant leaves is ground in 5 ml

7   of TRIzole (Life Technologies) to release nucleic acid.

8   2.   The homogenate is separated by centrifugation and

9   two clear supernatants of 1 ml each are collected. Each of

10 the supernatants is mixed with 600 μl of chloroform. 750 μl

11 of the aqueous solution is recovered from each tube after

12 centrifugation and is placed in a clean tube.

13   3.   750 μl of isopropanol is mixed with each solution

14 and the resulting solutions are kept at room temperature for

15 20 min to precipitate the nucleic acids.

16   4.   Nucleic acid in one tube (a) is harvested by

17 centrifugation (12,000 rpm) for 15 min at room temperature.

18 The pellet is dissolved in 75 μl of water after removal of

19 liquid and drying the pellet. 25 μl of the sample is diluted

20 1:1 with 25 μl of water and the resulting solution is

21 designated as A1 and the remaining 50 μl A2.

22   5.   Nucleic acid precipitated by isopropanol in the

23 other tube (B) is divided to 500 μl (B1) and 1000 μl (B2).

24 Nucleic acid in the two solutions are subjected to matrix

25 purification as described in Example 1 and 2. The nucleic

26 acid from each sample is dissolved for recovery in 50 μl

27 water.

28   6.   5 μl of each sample (A1, A2, B1, B2) may be

1   subjected to agarose g l electrophoresis (1.2%, 0.5 µg/ml

2   ethidium bromide).

3   7.   400 µl water is added to each sample and the

4   resulting solutions are examined by a UV spectrophotometer.

5   RNAs of small (tRNA) and large (rRNA) sizes are isolated

6   with the cellulose matrix, and DNA of very high molecular

7   weights is also present. The band patterns of the RNAs

8   obtained by the two methods are identical. Sample A2

9   contained the largest amount (114 µg) of nucleic acids (mainly

10 RNA) and sample A1 contains approximately half (62 µg) as much

11 as A2. Sample B1 has nearly the same amount (53 µg) of

12 nucleic acids as A1. Sample B2 recovers approximately 81 µg.

13   In this example, A1 and A2 are prepared by the complete

14 TRIzole method, and B1 and B2 by a modified method in which

15 the cellulose matrix method is used to replace high speed

16 centrifugation. Thus, the quality of the RNAs are expected to

17 be as good as that isolated by the complete TRIzole method.

18 The column procedure may be more reliable for isolating small

19 quantities of nucleic acids (20 µg) because pellet formation

20 is not required.

21   EXAMPLE 5

22 Demonstration of Cellulose for DNA Isolation and Concentration

23 From Liquid Whole Blood Samples Using the Suspension Format.

24   SETUP

25   Samples   1   2   3   4   5   6   7   8

26   Buffer

27   Extraction buffer: +   +   +   +   -   -   -   -

1   GEDTA: -   -   -   -   +   +   +   +

2   Matrix

3   Whatman cellulose: -   -   +   +   -   -   +   +

4   Procedure A: (Using SDS/proteinase-K-containing Extraction

5   buffer for nucleic acid solubilization)

6   1. Add 250 ul Extraction buffer and 12.5 ug proteinase K

7   to each 250 ul whole blood sample, total 4 samples (#1-#4).

8   2. The samples are incubated at 56°C for 1.5 hours.

9   3. Add 250 ul of 40% PEG and 12.5 ul of 5 M NaCl to each

10   sample; mix the samples for about 5 min.

11   4. Centrifuge the samples for 2 min at 2 k rpm and

12   recover the supernatant for each sample.

13   5. Add 5 mg Whatman cellulose to Samples 3 and 4 and add

14   250 ul 5 M NaCl to all the 4 samples; mix the samples for

15   about 5 min.

16   6. Centrifuge the samples for 2 min at 2 k rpm; save

17   pellets 1,2,3,4 and recover each supernatant; designate

18   supernatants as 1',2',3',4' respectively.

19   7. Centrifuge samples 1'-4' for about 5 min at 14 k rpm

20   to collect any precipitant; discard supernatant.

21   8. Add 1 ml washing solution to each of the samples #1,#2

22   and 1'-4'; after a gentle mixing, centrifuge the 6 samples

23   together as Step 7; the pellets are air-dried and each sample

24   is dissolved in 100 ul of elution buffer.

25   9. Cellulose fibers in samples 3 and 4, which look

26   reddish, are collected in independent matrix-collection

27   devices, washed twice with total 2 ml washing solution.

1   10. The collected cellulose is dried by spinning at 5 k

2 rpm for 2 min and DNA of each sample is eluted twice with a

3 total of 50 ul elution buffer. The resulted colorless DNA

4 solutions are finally adjusted to 100 ul for each.

5   11. 10 ul of each sample is analyzed in a 1% agarose gel.

6 Procedure B: (Using chaotrope-containing solution for nucleic

7 acid solubilization)

8   1. Add 250 ul GEDTA to each 250 ul whole blood, total 4

9 samples (#5-#8); mix the samples for 5 min.

10   2. Add 250 ul of isopropanol to each sample and 5 mg

11 Whatman cellulose to #7 and #8, mix the samples for 5 min.

12   3. Centrifuge the samples for 2 min at 2 k rpm; save the

13 pellets (5-8) and recover the supernatant (5'-8').

14   4. Centrifuge all the supernatants at 14 k rpm for 5 min;

15 air-dry the pellets and dissolve each pellet in 100 ul of

16 elution buffer.

17   6. Add 250 ul of water and 250 ul of GEDTA to cellulose

18 in #7 and #8, which look red; after mixing for 2-3 min, add

19 250 ul of isopropanol to each and mix again for another 2-3

20 min.

21   7. The cellulose fibers in sample #7 and #8 are collected

22 by a matrix collection device separately.

23   8. The fibers in a matrix collection device are washed

24 twice with total 2 ml washing solution; the fibers are then

25 dried by spinning at 5 k rpm for 2 min.

26   9. DNA associated with the cellulose fibers is eluted

27 twice in a total of 50 ul elution buffer. Each colorless DNA

1   solution is finally adjusted to 100 ul.

2   10. 10 ul of each sample is analyzed in a 1% agarose gel.

3   The results were as follows.

4   1. For samples that do not contact the cellulose, the

5   majority of the DNA remains in the first supernatant (1 and 2

6   vs. 1' and 2'; 5 and 6 vs. 5' and 6').

7   2. For samples that contact the cellulose, a significant

8   amount of DNA is in the pellet fraction after low speed

9   spinning (3,4,7,8), indicating that the precipitated nucleic

10   acid is associated with cellulose in suspension. We attribute

11   the nucleic acid observed in the supernatant fraction

12   (3',4',7',8')to fiber-DNA complexes that are carried over.

13   3. Procedure B is simpler than Procedure A. But GEDTA is

14   a more hazardous solution. A260nm/A280nm is about 1.8 for

15   sample #7 and #8.

16   4. Less total nucleic acid is recovered with Procedure A,

17   likely to be due to incomplete cell lysis under the described

18   conditions. Higher yields are observed when increased SDS

19   concentration is used for more diluted samples.

20   The conclusions were as follows.

21   1. Re-extracting the nucleic acid is a necessary step for

22   removal of pigmented and other contaminants.

23   2. Suspension format is a convenient way for the re-

24   extraction step.

25   3. Nucleic acids bind to cellulose efficiently in

26   suspension.

1   4. Nucl ic acid can be isolated in a concentrated form:

2   nucleic acid in >0.25 ml of blood can be concentrated to a

3   final volume of <50 ul.

4   5. The isolated DNA is pure: eluted DNA is colorless with

5   little protein contamination.

6   EXAMPLE 6

7   DNA isolation Using Agarose Matrix.

8   Solutions and reagents were the same as in Example 1

9   unless other specified. Dry agarose powder (FMC, Type LE) is

10   suspended in water at room temperature at a concentration of

11   approximately 5 mg/ml. Matrix is washed with water and matrix

12   solution before they are used for DNA isolation.

13   DNA sample preparation (1.75 μg of Salmon Testes DNA for

14   each sample) and isolation procedures are the same as

15   described in Example 1 and 2. Isolated DNAs are analyzed by

16   agarose gel and optical density measured. About 92% of DNA

17   can be recovered by the agarose matrix, compared to those

18   recovered by ethanol precipitation when the same amounts of

19   DNA are used.

20   EXAMPLE 7

21   DNA Isolation Using Synthetic Fiber Matrix.

22   Solutions and reagents are the same as in Example 1

23   unless otherwise specified. Synthetic cotton from a cosmetic

24   puffball (purchased from a local department store) is cut to

25   short fragments (1-2 mm).

26   Sample preparation, DNA isolation and analysis are the

1   same as described in Example 8. About 50% of DNA is recovered

2   with this matrix, compared to those recovered by ethanol

3   precipitation when the same amounts of DNA were used. This

4   relative low yield of recovery is expected due to the lower

5   hydrophobicity of this matrix compared to a cellulose matrix.

6   EXAMPLE 8

7   DNA Isolation Using Polyacrylamide Matrix.

8   Solutions and reagents are the same as in Example 1

9   unless otherwise specified. 7.5 ml of 30%

10   acrylamide/bisacrylamide solution and 7.5 ml of water were

11   mixed. a 15% polyacrylamide gel was formed and is broken into

12   fine particles (0.5-1.5 mm) mechanically. The suspension is

13   washed with water extensively until soluble materials and

14   unpolymerized acrylamide are removed.

15   Sample preparation and subsequent purification procedures

16   are essentially the same as described in Example 2. DNA is

17   recovered in basically the same yield by the polyacrylamide

18   matrix as by cellulose matrix.

19   A summary of recovery of nucleic acids, relative to a

20   100% recovery of nucleic acid using the phenol or silica

21   isolation methods described hereinabove, is provided in Table

22   I.

23   In Table I, the following key is used. Phenol refers to

24   phenol/chloroform extraction followed by ethanol

25   precipitation; Silica refers to a silica glass powder matrix

26   for DNA purification; CF refers to a cellulose fiber matrix

27   for DNA purification; Agarose refers to agarose powder matrix

1 for DNA purification; Synthetic refers to a synthetic cotton

2 fiber matrix for DNA purification; PAG refers to a

3 polyacrylamide gel suspension matrix for DNA purification.

4   In Table 1, the DNA referred to is as follows. Lambda:

5 lambda DNA; Salmon: salmon testes DNA; DBS: dried blood spot

6 DNA; DSP: double stranded plasmid DNA; SSP: single stranded

7 plasmid DNA; Plant: plant RNA. In addition, each number

8 refers to percentage of recovery, control is 100%; NA refers

9 to not applicable, or not available; + refers to the same or

10 nearly the same as controls.

TABLE I

Method	Form or source of nucleic acids					
	Lambda	Salmon	DBS	DSP	SSP	Plant
Phenol	100	100	100	NA	100	100
Silica	NA	NA	NA	100	NA	NA
CF	100	99	+	+	70-80	+
Agarose	NA	92	NA	NA	NA	NA
Synthetic	NA	50	NA	NA	NA	NA
PAG	NA	NA	+	NA	NA	NA

EXAMPLE 9

DNA Isolation Using Cellulose Coated Particles.

17   Solutions and reagents are the same as in Example 1

18 unless otherwise specified. The nucleic acid is first

1   solubilized and then aggregated by precipitation. Cellulose-

2   coated particles, e.g., magnetic beads, are added to the

3   aggregating buffer containing aggregated nucleic acid and the

4   aggregated nucleic acid is allowed to contact the cellulose

5   fibers on the beads for 2 or 3 minutes. The cellulose-coated

6   particles and associated nucleic acids are removed from the

7   solution by means commonly known in the art; in the case of

8   magnetic beads, a magnetic field is applied to draw the

9   nucleic acid away from the solution, which is then removed.

10   The magnetic field is then released. A wash solution is

11   applied and a magnetic field is applied. The magnetic field

12   is then released again. Elution of DNA from the beads is

13   accomplished by adding an aqueous buffer, applying the

14   magnetic field, and then removing supernatant-containing DNA.

15

16   Apparatuses of the Invention

17   As illustrated in Fig. 1, an apparatus of the invention

18   will include plural housings 100, 100' and a planar surface

19   support 104 for convenient simultaneous handling of the plural

20   housings. Each housing 100, 100' possesses an inlet 101, 101'

21   and an outlet 103, 103', and defines a flowpath 105, 105' for

22   flow of liquid therebetween. Housings 100, 100' contain

23   matrix 102, 102', as defined and described herein. The plural

24   housings and surface support 104 may be an integral unit, or

25   the housings may be separate from and adapted to fit into the

26   support 104. The housings may also include barrier means 106,

27   106' near the outlet end 103, 103', which barrier means serves

28   to pr vent matrix from exiting the outlet of the housing.

1   Th support 104 may be a plate or tray containing holes

2   into which the housings fit, or it may be a simple wire or

3   plastic rack.   The apparatus may optionally include a

4   collection surface 107 which is positioned beneath the support

5   surface 104.   The collection surface is also a planar surface

6   which includes sample collection sites 108, 108'. Sites 108,

7   108' may be simple indentations on the surface of a plastic

8   plate or they may be cups or tubes, e.g., microfuge tubes,

9   which fit into the plate. Collections sites 108, 108' may be

10   water insoluble, such as plastic, for collection of liquid

11   flowthrough from the housing, or they may be absorbent pieces

12   of filter paper for absorbing flowthrough. Sites 108, 108'

13   are for collecting liquid that flows through the housing,

14   whether it be matrix washings of unwanted material or eluted

15   nucleic acid.   During operation, the apparatus may include two

16   collection surfaces of the format of surface 107, a first

17   collection surface 106 for collection of unwanted materials

18   which flow through the housings, and the second collection

19   surface 107 for collection of eluted nucleic acid.   The

20   collection surface 107 may lie beneath surface 104; however,

21   optimally, surface 106 will fit snugly within the edges of

22   surface 104.   In the embodiment of the invention shown in Fig.

23   1, flow through may be collected using gravity flow or by

24   centrifugation of the entire apparatus, or by pressure applied

25   from top of the housing.

26   Alternatively, as shown in Fig. 2, the apparatus may

27   include dish 109, which may include means for connecting 110

28   the apparatus to a vacuum source to assist in washing the

1   matrix. a vacuum source may be connected to the vacuum

2   connecting means 110 and a vacuum applied to suck excess

3   solutions from the column. Vacuum connecting means 110 may

4   include a connector such as a compression fitting, ferrule,

5   coupling, or other structure known in the art capable of

6   accepting and holding a vacuum. Although gravity flow may be

7   used to pull liquid through the housing, use of a vacuum unit

8   expedites the method of the invention.

9   The apparatus shown in Fig. 2 also may be combined with

10   the apparatus shown in Fig. 1. That is, surface support 104,

11   containing plural housings 100, 100', and collection tray 107

12   may be used along with dish 109 such that the collection tray

13   107 fits snugly within dish 109. When vacuum is applied to

14   the apparatus via connecting means 110, the vacuum pulls

15   liquid through the housing, matrix, outlet, and onto the

16   collection sites 108, 108'.

17   In operation, plural biological samples in liquid form

18   are applied to the inlet 101 of the plural housings, whereupon

19   each sample flows along flow path 105 into and through the

20   matrix 102. Contact between nucleic acid in the sample and

21   the matrix results in binding of nucleic acid to the matrix.

22   Nucleic acid is thus retained, while unwanted components of

23   the biological sample flow through the matrix and screen 106,

24   and exit via outlet 103. The matrix may be washed prior to,

25   during, or after nucleic acid binding, if desired.

26   After binding, which may take no longer than a few

27   minutes, or the time interval in which the liquid sample flows

28   through the matrix, bound nucleic acid is eluted from plural

1   matrices simultaneously by dispensing lution buffer into the

2   plural housings and fitting the support plate 104 over the

3   sample collection tray 107. Centriguation and gravity may be

4   used to pull the elution buffer through the matrix (Fig. 1).

5   Alternatively, a vacuum source may be connected to dish 109

6   and support plate 104 may be placed over dish 109 to expedite

7   flowthrough (Fig. 2). If desired, support plate 104,

8   collection plate 107 and dish 109 may be sandwiched together

9   for simultaneous isolation of plural nucleic acid samples. If

10   desired, the unit can be modified so that liquid can flow

11   through the housing under pressure applied to 101 or 101', and

12   samples are collected via 108, 108'.

13   Another apparatus according to the invention for

14   isolation of substantially pure nucleic acid includes any

15   solid relatively inert organic surface such as, e.g., plastic,

16   an inorganic surface such as a metal surface, the surface

17   being coated with a matrix as described herein. An example of

18   preparation and use of a matrix-coated surface is provided

19   below.

20   For example, a polypropylene Column may be coated with

21   cellulose matrix as follows. 1 - 10 mg fibrous cellulose,

22   prepared as described herein is contacted with the activated

23   surface of the column; e.g., cellulose bound irreversibly to

24   the plastic. The amount of any type of matrix-coated on the

25   column will be that amount which is sufficient to bind nucleic

26   acid without a substantial amount of non-specifical binding.

27   Non-specific binding is that binding which occurs in the

28   absence of matrix-coating. Non-specific binding is not

1   substantial wh n less than 5% of nucleic acid is bound in the

2   presence of matrix. The matrix will be irreversibly bound to

3   the support surface, i.e., such that it is not lost from the

4   surface upon elution of the nucleic acid from the support.

5   In use, a biological sample containing nucleic acid is

6   contacted with the matrix-coated support under conditions

7   which permit the nucleic acid to bind, as taught hereinabove.

8   The support may then be washed and the nucleic acid eluted, as

9   taught herein.

10

1   OTHER EMBODIMENTS

2   Other embodiments will be evident to those of skill in

3   the art. Although the invention has been shown and described

4   with respect to an illustrative embodiment thereof, it should

5   be appreciated that the foregoing and various other changes,

6   omissions, and additions in the form and detail thereof may be

7   made without departing from the spirit and scope of the

       invention as delineated in the claims.

CLAIMS

1.   A method of isolating nucleic acid in a substantially purified form, said method comprising the steps of:

a) contacting a biological sample comprising nucleic acid with a matrix under conditions which permit said nucleic acid in said sample to bind to said matrix, said matrix comprising a solid hydrophilic organic polymer without an effective positive charge; and

b) recovering said nucleic acid from said matrix.

2.   The method of claim 1 wherein said conditions also permit said nucleic acid in said sample to aggregate.

3.   The method of claim 1, said matrix comprising a matrix suspension.

4.   The method of claim 1, further comprising the step of contacting said biological sample with a buffer under conditions sufficient to solubilize the nucleic acid.

5.   The method of claim 1, wherein said conditions comprise incubating said sample in a nucleic acid precipitating solution.

6.   The method of claim 1, further comprising the step, prior to step a) of aggregating said nucleic acid in said biological sample.

7.   The method of claim 5, wherein said solution compris s a
precipitating ingredient selected from the group consisting of
organic solvents, soluble organic polymers and salts.

8.   The method of claim 7, wherein the organic solvent may be
any one of isopropanol, ethanol, and acetone.

9.   The method of claim 7, wherein said organic polymer
consists essentially of polyethylene glycol.

10.   The method of claim 7, wherein said salt is one of Na˙ and
Li˙.

11.   The method of claim 1, wherein said matrix is selected
from a group consisting of polysaccharides and polypeptides.

12.   The method of claim 11, wherein said polysaccharides are
selected from the group consisting of agarose and chitin.

13.   The method of claim 3, said matrix comprising cellulose.

14.   The method of claim 11, wherein said polypeptides are
selected from the group consisting of leather, wool and silk.

15.   The method of claim 1, wherein said matrix is selected
from the group consisting of naturally occurring
polysaccharides and polypeptides, synthetic hydrophilic
polymers, and chemically modified polymers that are

hydrophobic polym rs which b come hydrophilic after ch mical
modification.

16.   The method of claim 1, wherein said matrix is coated on a
surface.

17.   The method of claim 16, wherein said matrix is coated on
an inorganic microparticle.

18.   The method of claim 3, wherein said matrix suspension
comprises cellulose-coated magnetic beads.

19.   The method of claim 1, wherein said matrix is a synthetic
gel.

20.   The method of claim 19, wherein said synthetic gel is
selected from the group consisting of polyacrylamide and
hydrogel.

21.   The method of claim 1, wherein said matrix is a synthetic
fiber.

22.   The method of claim 21, wherein said synthetic fiber is
selected from the group consisting of polyamides, polyesters,
polyacrylonitrile, and polyurethane.

23.   The method of claim 1, wherein said matrix is a synthetic
plastic.

24. The method of claim 23, wherein said synthetic plastic is selected from the group consisting of synthetic plastics including polycarbonate, phenol-formaldehyde resins, polysulfide, poly(vinyl butyryl), poly(vinyl chloride), poly(vinylidene chloride), poly(ethylene), and polystyrene.

25. The method of claim 21, wherein said synthetic fiber contains at least one polar group selected from the group consisting of hydroxyl, carboxyl, amino, and thiol.

26. The method of claim 18, wherein said synthetic plastic contains at least one polar group selected from the group consisting of hydroxyl, carboxyl, amino, and thiol.

27. A method of isolating nucleic acid in a substantially purified form, said method comprising the steps of:

a) contacting a biological sample comprising nucleic acid with a matrix-coated support under conditions which permit said nucleic acid in said sample to bind to said matrix, said matrix comprising a solid hydrophilic organic polymer without an effective positive charge; and

b) recovering said nucleic acid from said matrix.

28. The method of claim 27, said surface comprising a surface selected from the group consisting essentially of a plate, a well, a column, and a microtiter dish.

29. An apparatus for isolating nucleic acid in a

substantially purified form from multiple biological samples
simultaneously, said apparatus comprising:

a plurality of housings, wherein each said housing
comprises an inlet and an outlet and defines a flowpath for
flow of a sample therethrough, said flowpath comprising a
matrix comprising a solid hydrophilic organic polymer without
an effective positive charge, and

support means for holding said plurality of housings in
place such that nucleic acid in said plural biological samples
may be isolated simultaneously.

30.   The apparatus of claim 29, wherein each housing of said
plurality comprises a barrier means to allow flow of liquid
along the flowpath and through the housing outlet, and to
prevent the matrix from exiting the housing via the outlet.

31.   The apparatus of claim 30, wherein each said housing
comprises a lower portion leading to the outlet, and said
barrier means is positioned within the lower portion of the
housing.

32.   The apparatus of claim 29, further comprising a connector
for connecting said plurality of housings to a vacuum or
pressure source.

33.   The apparatus of claim 29, further comprising a
collection tray for simultaneously collecting plural nucleic
acid samples.

34. An apparatus for isolating nucleic acid in a
substantially purified form from a biological sample,
comprising;

a support surface containing an irreversibly bound
matrix, said matrix comprising a solid hydrophilic organic
polymer without an effective positive charge.

35. The apparatus of claim 34, said support surface
comprising a cylindrical housing.

36. The apparatus of claim 34, said support surface
comprising a multi-well plate.

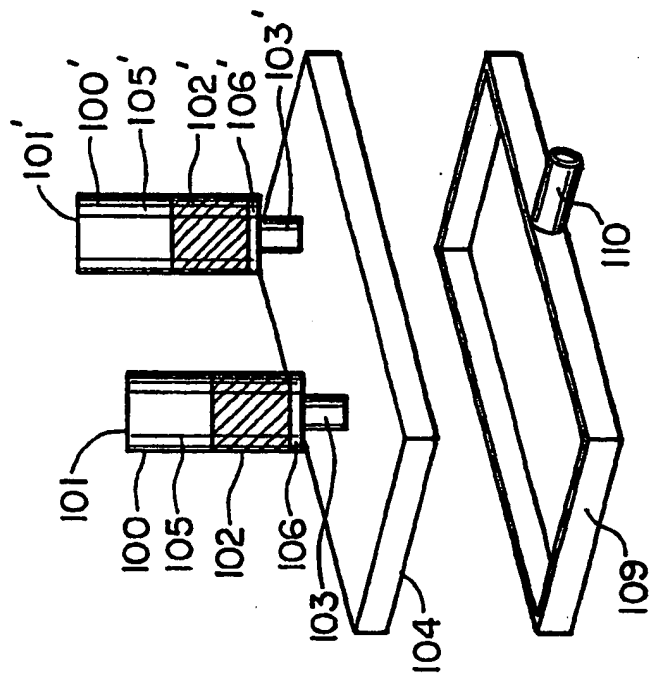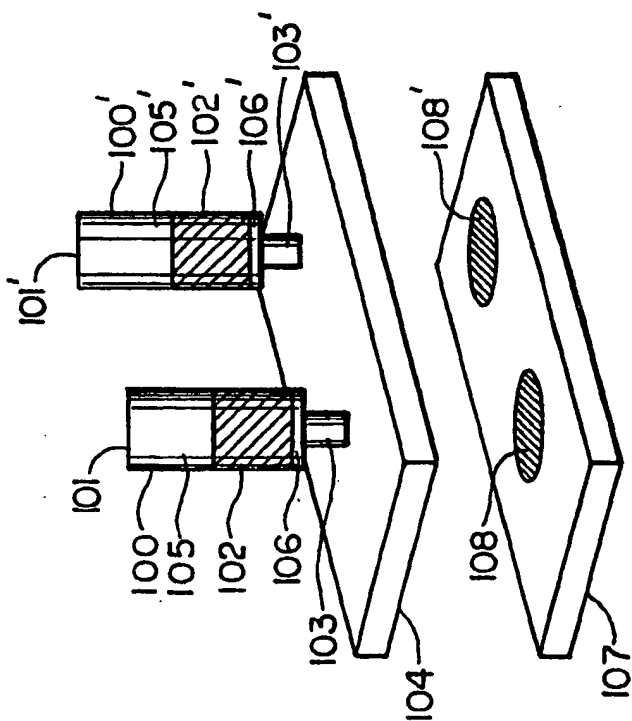37. The apparatus of claim 29 or 34, said matrix comprising
cellulose.

FIG. 2

FIG. 1